Technical Documentation to Support Development of Minimum Flows and Levels for the Caloosahatchee River and Estuary

Appendix B

Effects of seasonal and water quality parameters on oysters (*Crassostrea virginica*) and associated fish populations in the Caloosahatchee River.

Contents

Progress Report submitted
to
Dr. Peter Doering

July 2002.

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Abstract

Disease prevalence (% infected oysters) and intensity of oyster pathogen, *Perkinsus marinus*, were investigated at five locations (Piney Point, Cattle Dock, Bird Island, Kitchel Key and Tarpon Bay) in the Caloosahatchee Estuary in relation to season and freshwater releases (i.e., salinity) from Lake Okeechobee. Ten oysters per month were analyzed from each sampling location during September 2000 - June 2002. Data were analyzed as year 1 (September 2000 - August 2001) and Year 2 (September 2001 - June 2002). Freshwater releases > 300 cubic feet per second (CFS) from Lake Okeechobee by the South Florida Water Management District (SFWMD) during dry months (Nov - May) in year 2 resulted in lower salinities at all locations compared to year 1. Freshwater releases during the dry months in Year 1 were less than 300 CFS. Salinities during sample collection were regressed against monthly average of 30-day moving average flow to predict salinity changes at the sampling locations. Results suggest that freshwater releases of 1000 CFS from Lake Okeechobee may decrease salinities at the sampling locations by 3.6 - 6 ppt (downstream - upstream locations) from respective prevailing salinities. Salinity, and temperature during the study period ranged from 3 - 40 ppt and from 16 - 32ºC respectively. Prevalence of *P. marinus* ranged from 0 - 90% while the intensity of infection ranged from 0 - 2.5 (on a scale of 0 - 5). Concomitant with higher freshwater releases and lower salinities at all sampling locations in year 2, intensity of *P. marinus* infection in oysters was significantly lower during year 2 compared to year 1. Infection intensity was also significantly different between sampling locations. It should be noted that while the prevalence of infection was high, overall infection intensities at various sampling locations were light (0.170 - 0.753) during both years suggesting that decreased freshwater releases less than 300 CFS (and higher salinities) did not result in lethal (heavy) infection intensities. Flows between 500 and 2000 CFS will result in optimum salinities for oysters and will result in sustaining and enhancing oyster populations in the Caloosahatchee Estuary. Data suggests that well-timed fresh water releases into Caloosahatchee River may prevent or lower *P. marinus* infections to non-lethal levels (light) in oysters and enable them to survive longer. Effects of high freshwater releases (and lowered salinities) on the condition index, recruitment, gonadal index, and growth of juvenile oysters are being examined in a series of field and laboratory experiments. The use of adaptive management approaches involving freshwater releases to sustain and enhance oyster populations is valuable to the ecology of the Caloosahatchee Estuary.

Introduction

A fundamental management goal of the Watershed Research and Planning Department is to “Protect, Enhance, and Rehabilitate Estuarine Ecosystems”. Using a suite of responses from Valued Ecosystem Component (VEC) species - oysters - the effects of freshwater releases from Lake Okeechobee were assessed. VEC species are those that sustain the ecological structure and function of dominant estuarine communities. These species provide not only food, but also the physical habitat utilized by other organisms for living space, refuge, and foraging sites. Examples of dominant estuarine communities are oyster bars and grass beds, with prominent species being the American oyster, *Crassostrea virginica* and the submerged aquatic vegetation (SAVs), *Vallisneria americana*, *Halodule wrightii*, and *Thalassia testudinum*. Historically, grass beds and osyter reefs have been dominant components of the Caloosahatchee estuarine system. Both habitat types still exist in the system today.

Oysters not only represent an important fisheries species commonly found in estuaries of the Atlantic and Gulf coasts of the U.S., but they are important ecologically. Individual oysters filter 4-34 liters of water per hour, removing phytoplankton, particulate organic carbon, sediments, pollutants, and microorganisms from the water column. This filtration process results in greater light penetration immediately downstream, thus promoting the growth of submerged aquatic vegetation. Although oysters assimilate 70% of the organic matter filtered, the remainder is deposited on the bottom where it provides food for benthic organisms. This secondary production, combined with a complex, three-dimensional, reef structure serving as nesting habitat and/or refuge, attracts numerous species of invertebrates and fishes (e.g., blue crab, mud crabs, grass shrimps, penaeid shrimp, blennies, gobies, killifishes, skilletfish, toadfishes). Furthermore, many of these organisms serve as forage for important fisheries species, birds, and mammals. Oysters are not only an important fisheries species, but oyster reefs serve as essential fish habitat. Due to their sessile nature, oysters make excellent candidates to investigate cause and effects relationship in examining watershed alteration effects. Due to the ecological role of oysters, their protection and restoration should therefore be a focus of resource managers.

The protozoan parasite, *Perkinsus marinus* has devastated oyster populations in the Atlantic (Burreson and Ragone-Calvo 1996), where it is currently the primary pathogen of oysters, and in the Gulf of Mexico (Soniat 1996). Andrews (1988) estimated that *P. marinus* can kill ~80% of the oysters in a bed. The distribution and prevalence of *P. marinus* is influenced by temperature and salinity with higher values favoring the disease organism (Burreson and Ragone-Calvo 1996, Soniat 1996, Chu and Volety 1997).

While the South Florida Water Management District (SFWMD) has conducted considerable research on SAV (Chamberlain and Doering 1998a, Chamberlain and Doering 1998b, Kraemer et al. 1999), studies involving other valued ecosystem components, such as oyster reefs, that occur in the higher salinity waters of the lower Caloosahatchee Estuary are presently lacking, but clearly necessary. To our knowledge, this project represents the first study of oysters in the Caloosahatchee River and will provide critical information for use in applying the VEC approach. The ultimate goal of this project is examine the effect of minimum flows and levels of freshwater into the Caloosahatchee Estuary and to provide target conditions for watershed management in the Caloosahatchee River that will sustain and enhance oyster populations.

This report summarizes the results of *Perkinsus marinus* infection prevalence and intensities in oysters from Caloosahatchee River during September 2000 - June 2002, focusing on the dry months (November - May). The overall objectives of the project were to evaluate the effect of season and spatial variation on condition and health of oyster populations in the Caloosahatchee and to determine the suitability of oyster habitat to crustaceans and fishes in relation to oyster health and condition. Results related to spat recruitment potential, and habitat suitability of oyster reefs for crustaceans and fishes in the Caloosahatchee River will be addressed in the next report.

Materials and Methods

Sampling Locations: Monthly water quality measurements and oyster collections were made at Piney Point (PP, 4 km upstream from river mouth), Cattle Dock (CD, 2 km upstream from river mouth), Bird Island (BI, 4 km downstream from river mouth), Kitchel Key (KK, 6 km downstream of river mouth), and Tarpon Bay (TB, 12 km downstream of river mouth).

Freshwater Releases and water quality: Data on freshwater releases from Lake Okeechobee via S-79 locks were obtained through continuous water quality monitoring by SFWMD (courtesy of Dr. Peter Doering and Ms. Kathy Haunert). Monthly means of the 30-day moving average flow (in cubic feet per second; CFS) were obtained from September 2000 - May 2002. Salinities and temperatures at sampling locations were noted during monthly samplings. Relationship between flows from S-79 locks and salinities at various sampling locations were assessed by regression analyses. Since salinities observed at the sampling locations included freshwater dilution due to rainfall and sheetflow, influence of these two factors could not be separated in the analyses.

P. marinus prevalence and intensity: Ten oysters from each of the five sites were assayed monthly for the prevalence (% infected oysters) and intensity of infection of *P. marinus* using Ray's fluid thioglycollate medium technique (Ray 1954, Volety et al. 2000). The intensity of infections were recorded using a scale in which 0 = no infection, 1 = light, 2 = light - moderate, 3 = moderate, 4 = moderate - heavy, 5 = heavy. Three-way ANOVA was used to detect the differences in *P. marinus* infection intensities due to sampling year / flow (no flow (<300 CFS) vs. low flow (>300 CFS)), sampling month, and sampling location.

Statistical anayses: Relation between freshwater releases at S-79 and salinities at various sampling locations were analyzed using correlation and regression analyses. Effect of sampling year, sampling month (season), and sampling location on *P. marinus* intensity were examines using a three-way ANOVA. When significant differences in means were observed, a multiple comparison test (Dunnett's T-3) was used assuming unequal variance.

Results

Water quality parameters: Temperature, salinity and freshwater flow (releases from S-79 were investigated during the study period. Mean monthly 30-day moving average flow at S-79 ranged from a minimum of 0.7 CFS in March 2001 to a maximum of 3813 CFS in September of 2001 (Fig. 1). Freshwater releases from S-79 were also higher during the dry months of year 2 (> 300 CFS) compared to year 1 (< 300 CFS). In general, freshwater releases were high in the summer months (July - October) and low during the dry / winter months (November to June). There was a significant inverse correlation between flows at S-79 and salinities at all sampling locations (65 - 76% correlation; $P < 0.0001$). Salinity at all locations decreased with increasing freshwater flows. Regression analyses for each site indicated that there was a highly significant relation between freshwater flow and salinities at all stations (Table 1). These regressions explained 43 - 58% of the variation (Fig. 2). Shell Point was considered as the river mouth (Chamberlain and Doering 1998a, b). Results suggest that for every 1000 CFS released at S-79, salinities at PP, CD, BI, KK, and TB would decrease by 6, 5.7, 5.3, 4.3, and 3.6 ppt, respectively from their ambient salinities (Table 1). According to our model, at zero flow, salinities at PP, CD, BI, KK, and TB would be 28.5, 29.9, 32.7, 33.5, and 36.6 ppt respectively (Table 1). Since observed salinities at these locations would also be influenced by sheet flow resulted by rainfall, and tides, effect of rainfall / sheet flow and tides could not be separated from the model. However predicted salinity at these locations are in very close agreement (± 3 ppt) with those predicted by Bierman's model (1993). Temperature during the study period ranged from 18 - 31ºC (Fig. 3). With the exception of Jan - Feb 2002, temperatures in corresponding months during years 1 and 2 were similar (<4ºC difference; Fig. 3). Salinities at all sampling locations were higher during year 1, a period of no flow - low flow, compared to year 2 where flows were higher than 300 CFS (Figs. 4 - 8).

P. marinus prevalence: Similar to salinities, prevalence of *P. marinus* in oysters from all locations was lower during dry months of year 2 compared with those from year 1 (Fig. 9 - 14; Table 2, $P < 0.0001$). The differences in prevalence between years, as expected, was more pronounced at upstream locations compared to the downstream location (TB). Prevalence of *P. marinus* infection during the dry months in first and second years at the sampling locations were as follows: PP - year 1, 20 - 40%; year 2, 0 - 11%, CD -year 1, 20 - 90%; year 2, 11 - 90%, BI - year 1, 13 - 90%; year 2, 0 - 60%, KK - year 1, 0 - 80%; year 2, 0 - 40%, and, Tarpon Bay - year 1, 0 - 50%; year 2, 0 - 50%.

P. marinus intensity: Intensity of *P. marinus* infections in oysters were calculated as weighted prevalence. This procedure incorporates the prevalence of infection and intensity of infections in individual oysters in calculating a weighted prevalence. Concomitant with salinities and freshwater flows, and similar to prevalence of *P. marinus*, intensity of infections in oysters from all sampling locations except the downstream station Tarpon Bay, was lower during year 2 compared to those in year 1

(Figs. 15-20; Table 2, P < 0.0001). Infection intensities during the dry months in first and second years at the sampling locations are as follows: PP - year 1, 0.2 - 0.4; year 2, 0 - 0.11, CD -year 1, 0.2 - 2.4; year 2, 0.1 - 1.5, BI - year 1, 0.2 - 2.5; year 2, 0 - 0.6, KK - year 1, 0 - 0.8; year 2, 0 - 0.6, and, Tarpon Bay - year 1, 0 - 0.5; year 2, 0 - 1. These results suggest that with the exception of CD and BI locations, oysters from all locations, during both no flow - low flow (year 1; < 300 CFS), and low flow - intermediate flow (year 2; > 300 CFS) had light infections that are non-lethal. Typically, intermediate - heavy infections (intensity 3 - 5) are considered lethal.

Summary and conclusions

Past studies demonstrated that low salinities (<12 ppt) retarded *P. marinus* infections in oysters (Ray 1954, Andrews and Hewatt 1957, Chu et al. 1993, Ragone and Burreson 1993, Chu and Volety 1997). Our field study demonstrates the relation between varying salinities and disease prevalence and intensity in oysters in the field. Despite the high prevalence of infection in oysters (0 - 90%), disease intensity is low due to a combination of factors -- temperature and salinity acting antagonistically resulting in low intensities (light infections). Given the flow rates from S-79, based on our model and that of Bierman (1993), salinities at all locations would have encountered salinities of 6 - 12 ppt, values that would retard development of *P. marinus* infections. The upstream station, PP, had the lowest infection intensities in oysters and lowest salinities. Higher infection intensities in oysters from CD may be as a result of the water quality and high boat wakes. This site receives water output from the City of Cape Coral and nearby marinas. As mentioned earlier, higher temperatures and salinities favor *P. marinus*. In the Caloosahatchee estuary, when summer temperatures reach as high as 32ºC, *P. marinus* infection prevalence and intensity should be high. However, during summer months, the combination of freshwater releases by SFWMD and high rainfall decreases the salinities to 0 - 12 ppt, depending on the station, keeping infection levels low. Similarly, during winter, when freshwater releases are none - low, and rainfall is lacking, salinities are high (30 -40 ppt). These high salinities should result in heavy *P. marinus* infections in oysters. However, during the winter months, temperatures are lower (15 - 18ºC) resulting in low infection levels despite high salinity. Temperatures and salinities in Caloosahatchee estuary act antagonistically keeping *P. marinus* infections at low levels. Similar decreases in *P. marinus* intensities in oysters concomitant with decreased salinities were observed in other southwest Florida estuaries (Thurston et al. 2001, Volety et al. 2001a, b). However, it has to be cautioned that high flow (> 3000 CFS) freshwater releases during summer time may have negative impacts on oyster populations.

Although oysters tolerate salinities between 0 - 42 ppt, growth is best achieved at salinities of 14 - 28 ppt; slower growth, poor spat production, and excessive valve closure occur at salinities below 14 ppt (see Shumway 1996). Battaller et al. (1999) reported lower growth and condition index of oysters grown at a low salinity site compared to a high salinity site in Canada. Similar results are seen in our current study (results not shown). Since the metabolic energy remaining after reproduction and daily maintenance is converted to biomass, an oyster stressed either by its water quality or by disease has less energy for growth and reproduction. In addition, oyster larvae respond to water flow, salinity, temperature and a host of chemical cues from adult oysters, hard substrates, and old oyster shells colonized by bacteria. The net result is that oyster larvae typically settle more frequently in areas of low flushing, higher salinities, and a dense accumulation of adults. In contrast, low salinities result in poor spat settlement and lower growth rates (Shumway 1996). Sudden changes in water quality and resulting poor oyster health may cause a shift in patterns of recruitment and survival. Either of these responses have significant impacts on recruitment of spat into the populations.

Oysters in the Caloosahatchee estuary reproduce between May and October (see previous progress report), a period that coincides with heavy rainfall and freshwater releases in excess of 4000 CFS. According to our model, as well as Bierman's model, these flows and rainfall will depress the salinity at all sampling locations to 4 - 15 ppt for extended periods (2-3 months). Lower salinities not only impact the survival, but also high flow flushes out the oyster spat produced during this period from the estuary into the ocean where suitable substrates for attachment are lacking. In fact, our laboratory experiments indicate that salinities < 5 ppt for more than 2-4 weeks would result in 80% mortality of oysters (see previous report). Given that flows in the Caloosahatchee River exceeded 3000 CFS during August - October 2001, salinities in all the sampling locations would have been between 2 - 15 ppt, conditions that are stressful to oysters and oyster spat. In addition, as a result of these high flows, large amount of spat would have been flushed into the Gulf of Mexico resulting in poor settlement.

In conclusion, under the current freshwater release regime and seasonal patterns, antagonistic effects of temperature and salinities keep *P. marinus* infection in oysters at low levels in the Caloosahatchee River. Freshwater releases from Lake Okeechobee during the dry months in year 1 were none - low (< 300 CFS) compared to year 2 when water releases were higher than 300 CFS (Fig 1). Lower salinities at all stations corresponding with freshwater releases indicate that salinities were influenced by the water releases by SFWMD (Correlation 65 - 76%). While the infection levels in oysters are lower in the dry months of year 2, compared to year 1, overall infections are light (Figs 15 - 20). These results suggest that flows < 300 CFS, do not cause "significant harm" as measured by *P. marinus* infections in the Caloosahatchee oyster populations. It has to be cautioned that the current study did not examine the effects of marine predators (oyster drills, crown conchs, whelks etc.) that dominate high salinity waters. Given that optimum salinity for oysters ranges from 14 - 28 ppt, under the prevailing salinity regimes, high flows exceeding 3000 CFS may cause severe mortality and low spat recruitment into the system. Flows between 500 CFS and 2000 CFS would result in salinities of 16 - 28 ppt at all stations. Under the current water management practices, oysters in the Caloosahatchee River are not stressed by low flows (< 300 CFS), but are stressed due to high flows exceeding 3000 CFS for extended periods (2 - 4 weeks).

Given our laboratory and field studies, a single freshet event (< 3 ppt), lasting up to 2 weeks will not have any significant effect on the mortality of oysters. While flows above 300 CFS resulted in lower disease intensities in all sampling locations, intensities under low flows (< 300 CFS), resulted in overall low - moderate non-lethal infections. Flows between 500 and 2000 CFS will result in optimum salinities for oysters and will result in sustaining and enhancing oyster populations in Caloosahatchee Estuary. The use of adaptive management approach involving freshwater releases to sustain and enhance oyster populations is invaluable to the ecology of the Caloosahatchee Estuary.

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Table 1: Model to predict relation between S-79 flows and salinities at sampling locations in Caloosahatchee Estuary.

Sampling station	Location in River (from the mouth)	Regression Equation	R-Sq %	P-value	Predicted salinity at zero flow	Predicted change in ambient salinity per 1000 CFS release at S-79
Piney Point	4 km upstream	Salinity = -0.006*flow + 28.49	58.1	0.0000	28.49 ppt	6.0 ppt
Cattle Dock	2 km upstream	Salinity = -0.006*flow + 29.88	54.2	0.0000	29.88 ppt	5.7 ppt
Bird Island	4 km downstream	Salinity = -0.005*flow + 32.67	48.0	0.0000	32.67 ppt	5.3 ppt
Kitchel Key	6 km downstream	Salinity = -0.004*flow + 33.51	42.8	0.0000	33.51 ppt	4.3 ppt
Tarpon Bay	12 km downstream	salinity = -0.004*flow + 36.53	57.5	0.0000	36.53 ppt	3.7 ppt

Table 2: Analyses of variance of *P. marinus* disease intensity in oysters from Caloosahatchee Estuary.

Source	Type III SS	df	Mean Square	F	Significance (P)
Station	28.15	4	7.04	16.99	0.000
Month	22.99	6	3.83	9.26	0.000
Year	11.85	1	11.85	28.60	0.000
Month*Station	23.35	24	0.97	2.35	0.000
Year*Month	4.55	4	1.14	2.75	0.028
Station*Month	27.27	6	4.54	10.98	0.000
Station*Month*Year	50.3	24	2.10	5.06	0.000

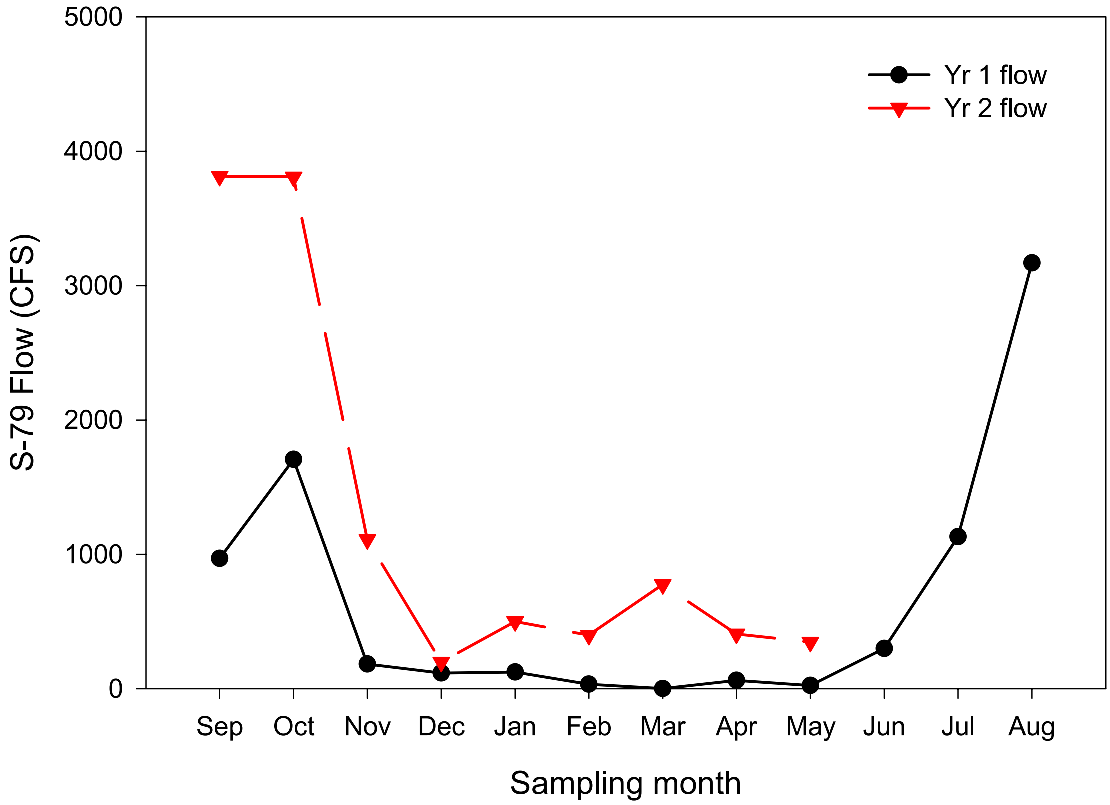

Fig. 1: Flow (in CFS) at S-79 in Caloosahatchee River during years 1 and 2. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Flow rates (< 300 CFS) were observed during winter / dry months (Nov - May) in Year 1 compared to Year 2.

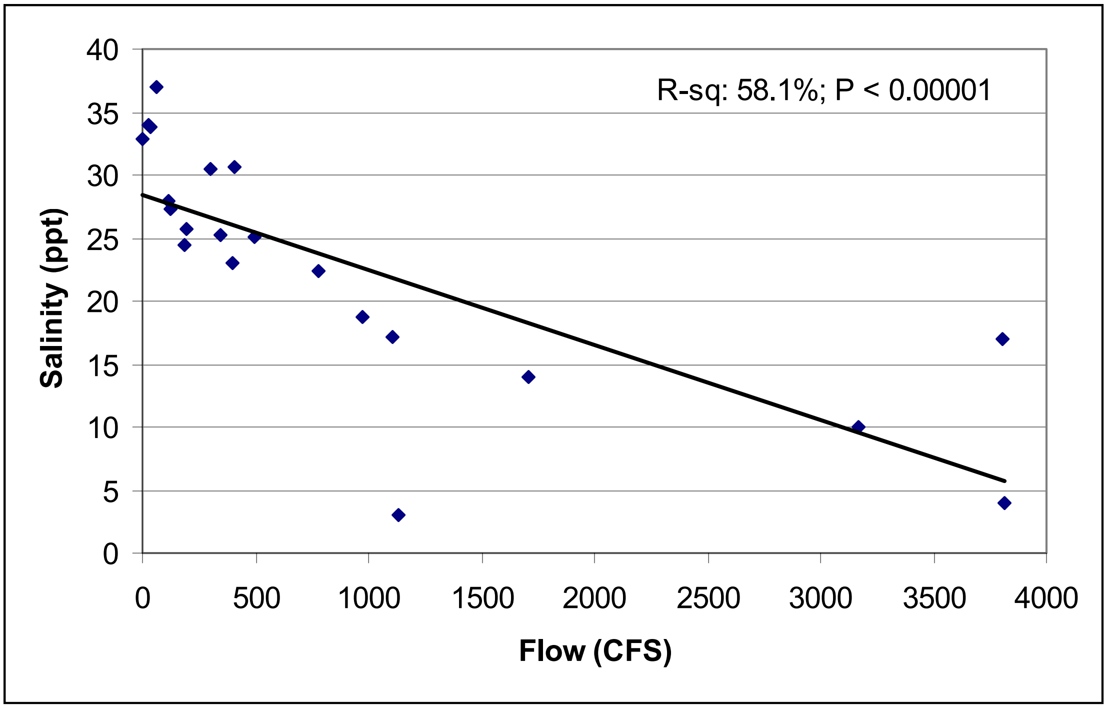

Fig. 2: Relation between S-79 flow (in CFS) and salinity at the upstream station, Piney Point, in Caloosahatchee River. Monthly average of 30 day moving average of flow at 79 was regressed against observed salinity (during sampling) at Piney Point. Effect of sheet flow and rainfall on the salinity in the sampling locations was not included in the regression model. Regression equation was as follows: Salinity = -0.006 x flow in CFS + 28.49. These results suggest that a flow of 1000 CFS at S-79 locks would result in a decrease of 6 ppt at Piney Point. Similar regression equations were constructed for Cattle Dock, Bird Island, Kitchel Key and Tarpon Bay.

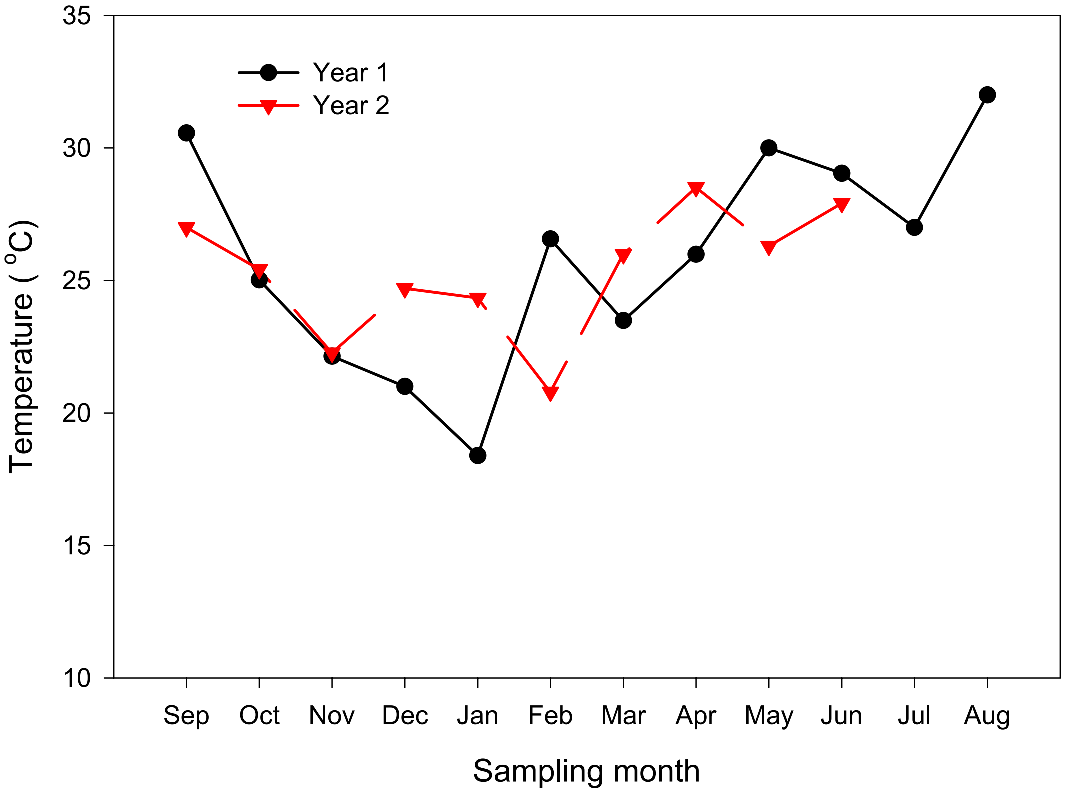

Fig. 3: Temperature (ºC) at Piney Point in Caloosahatchee River during years 1 and 2. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Temperatures were similar at all sampling locations (±1ºC). As expected, temperatures were lower in winter and spring months (Nov-Apr) compared to summer and Fall months.

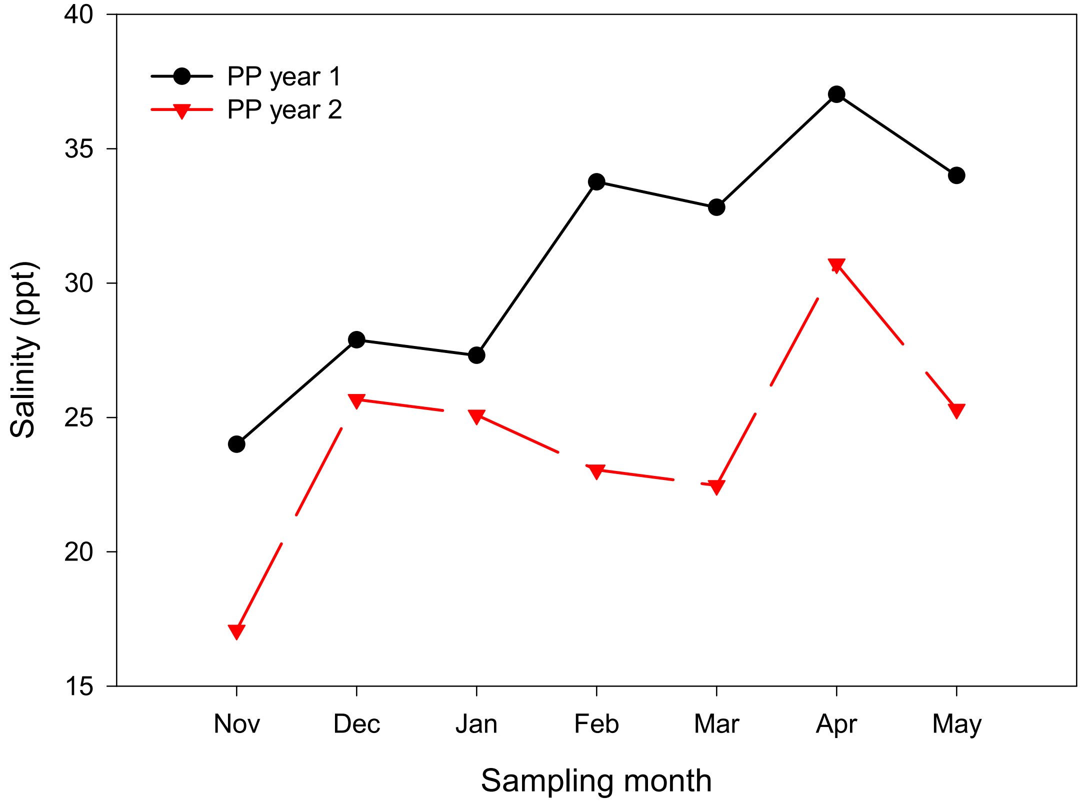

Fig. 4: Salinity (ppt) at Piney Point (PP) in Caloosahatchee River during dry months in years 1 and 2. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Salinities at all stations decreased with increased flow from S-79 locks. In addition, salinities in year 2 were lower than those in year 1 due to freshwater releases from Lake Okeechobee during year 2.

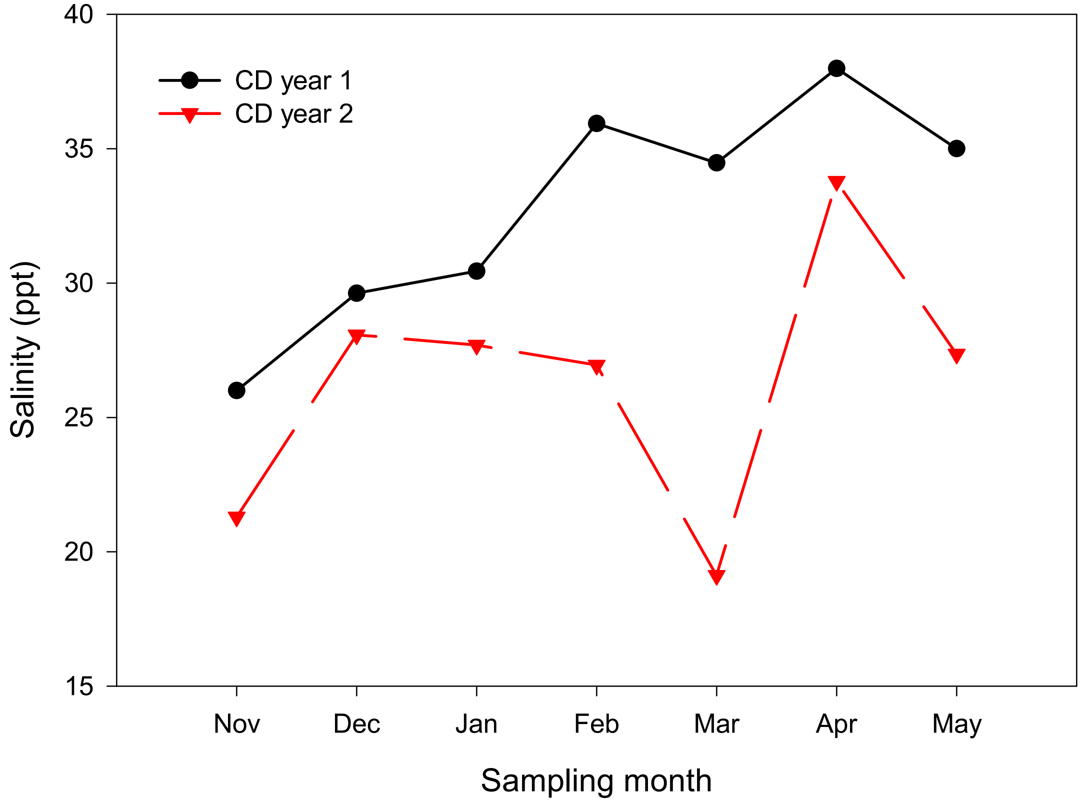

Fig. 5: Salinity (ppt) at Cattle Dock (CD) in Caloosahatchee River during dry months in years 1 and 2. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Salinities at all stations decreased with increased flow from S-79 locks. In addition, salinities in year 2 were lower than those in year 1 due to freshwater releases from Lake Okeechobee during year 2.

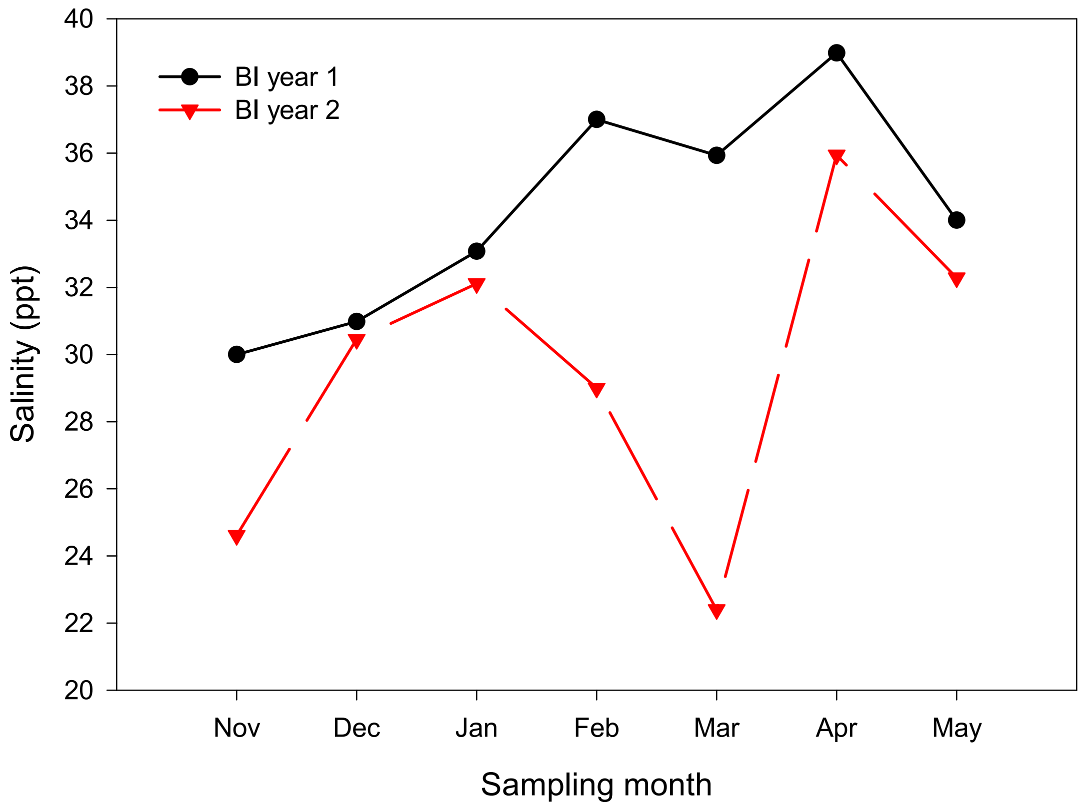

Fig. 6: Salinity (ppt) at Bird Island (BI) in Caloosahatchee River during dry months in years 1 and 2. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Salinities at all stations decreased with increased flow from S-79 locks. In addition, salinities in year 2 were lower than those in year 1 due to freshwater releases from Lake Okeechobee during year 2.

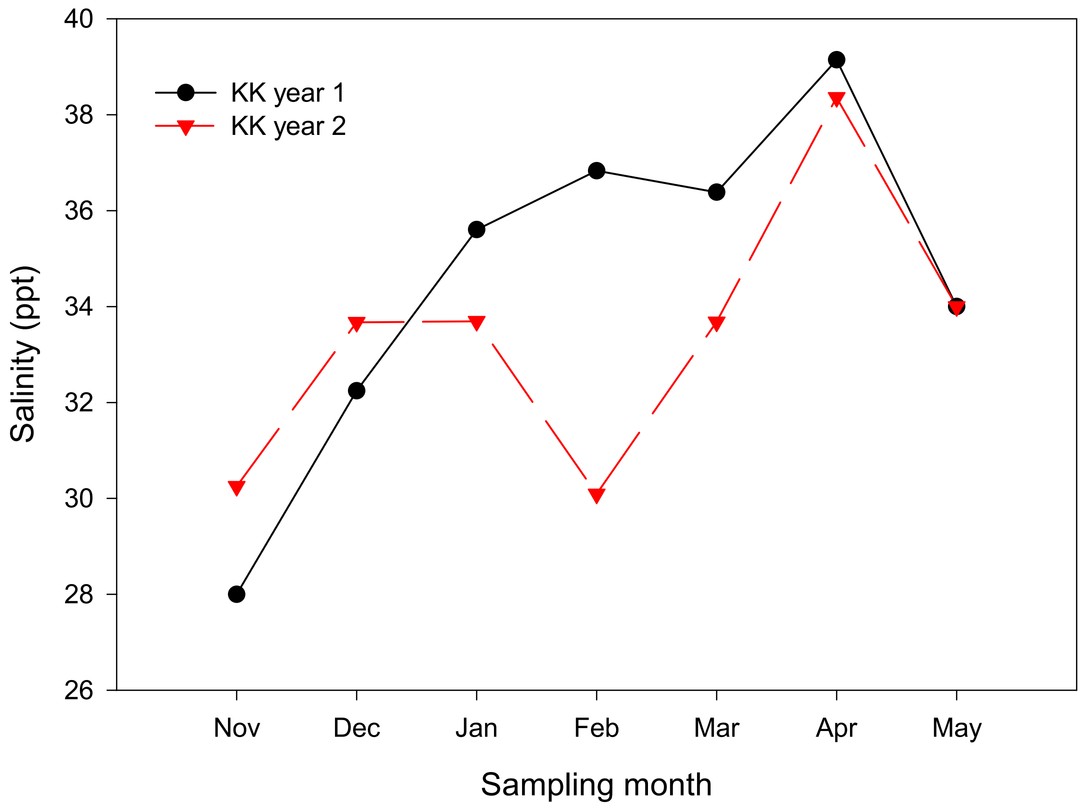

Fig. 7: Salinity (ppt) at Kitchel Key (KK) in Caloosahatchee River during dry months in years 1 and 2. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Salinities at all stations decreased with increased flow from S-79 locks. In addition, salinities in year 2, with the exception of Nov - Dec, were lower than those in year 1 due to freshwater releases from Lake Okeechobee during year 2.

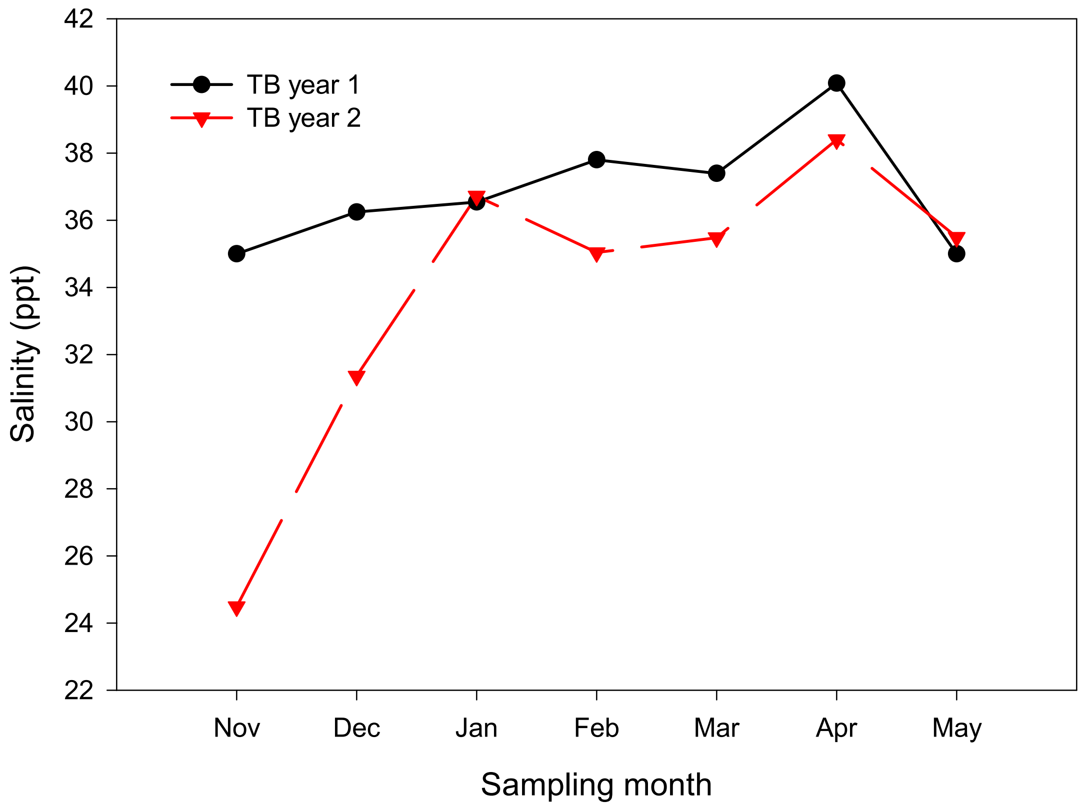

Fig. 8: Salinity (ppt) at Tarpon Bay (TB) in Caloosahatchee River during dry months in years 1 and 2. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Salinities at all stations decreased with increased flow from S-79 locks. In addition, salinities in year 2 were lower than those in year 1 due to freshwater releases from Lake Okeechobee during year 2.

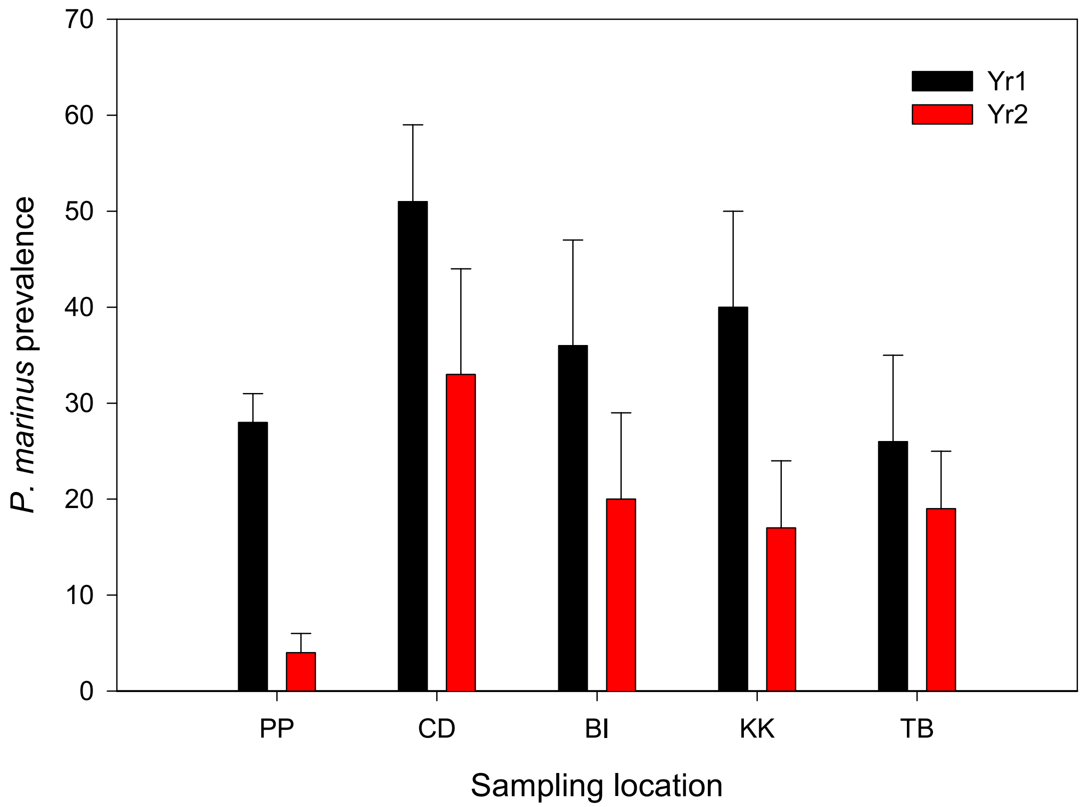

Fig. 9: Mean *P. marinus* prevalence (± SE) during winter months in oysters from Piney Point (PP), Cattle Dock (CD), Bird Island (BI), Kitchel Key (KK), and Tarpon Bay (TB) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Ten oysters per month were randomly samples from the sampling locations per month and prevalence of *P. marinus* in oysters was analyzed according to Ray 1954. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities resulted in lower infection intensities in oysters from all upstream locations.

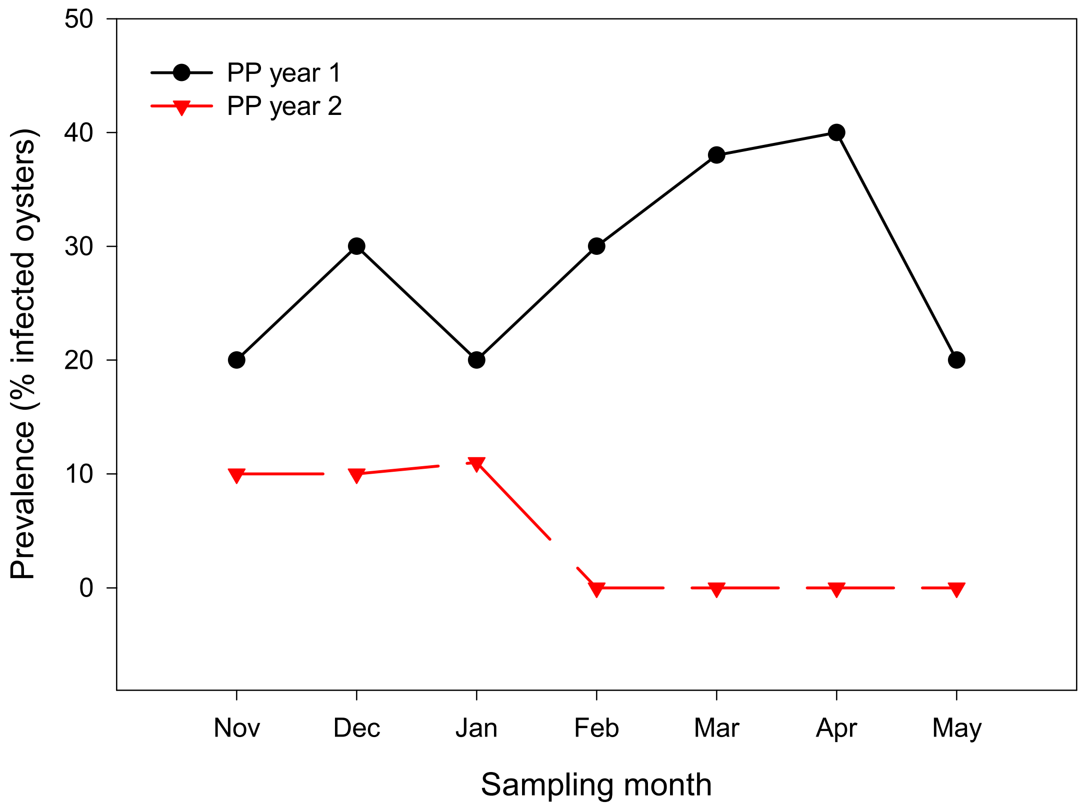

Fig. 10: Mean *P. marinus* prevalence (± SE) during winter months in oysters from Piney Point (PP) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters.

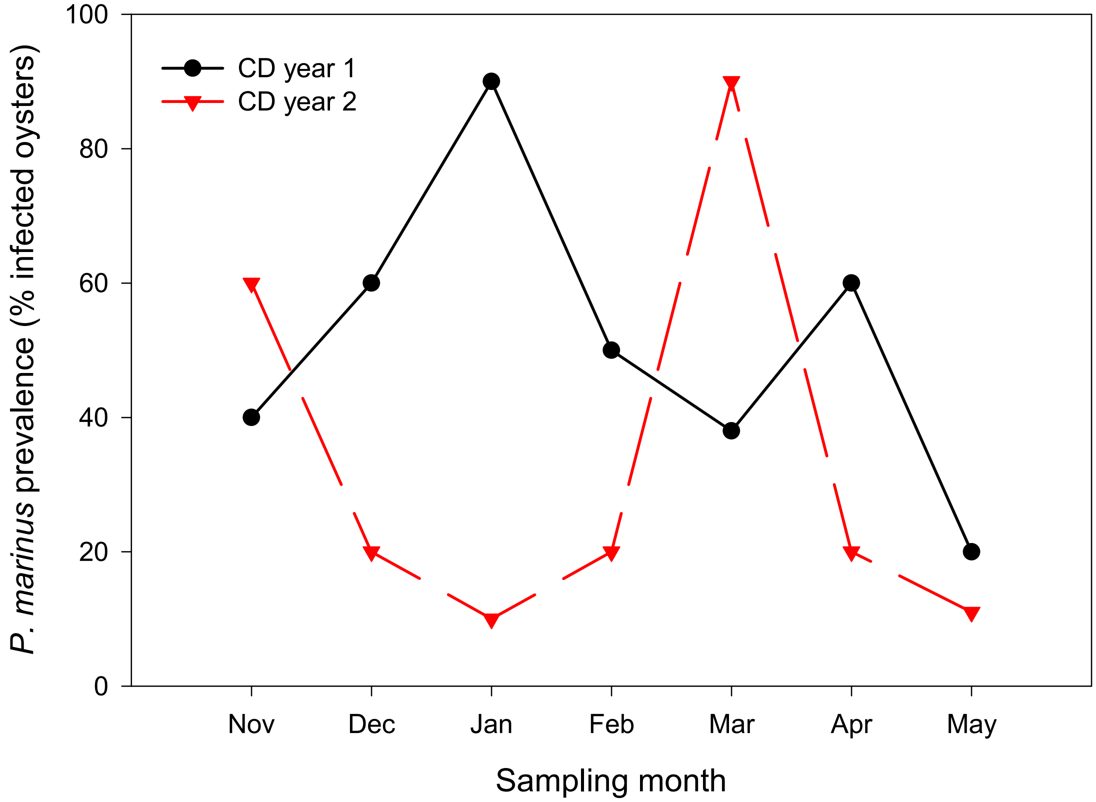

Fig. 11: Mean *P. marinus* prevalence (± SE) during winter months in oysters from Cattle Dock (CD) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters. Cattle Dock site also receives runoff water from the City of Cape Coral and nearby marinas.

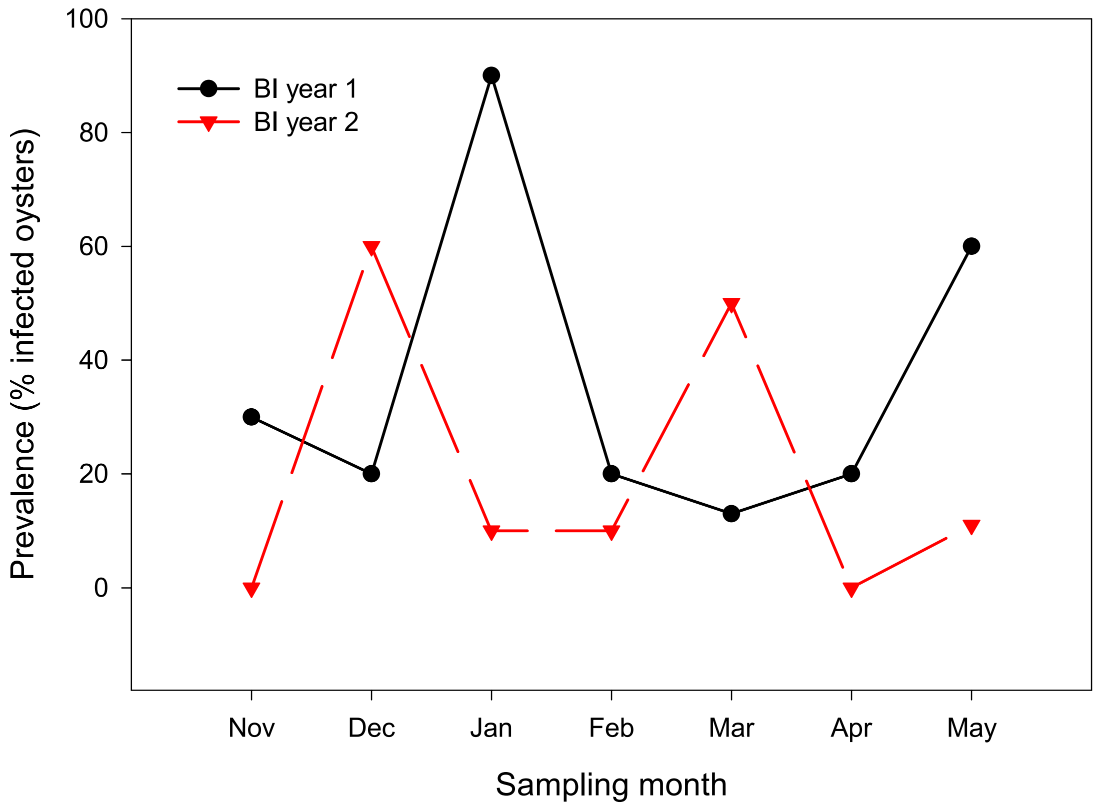

Fig. 12: Mean *P. marinus* prevalence (± SE) during winter months in oysters from Bird Island (BI) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters, however, due to the proximity of this station to marine environment and higher salinities, effects of freshwater releases are less pronounced.

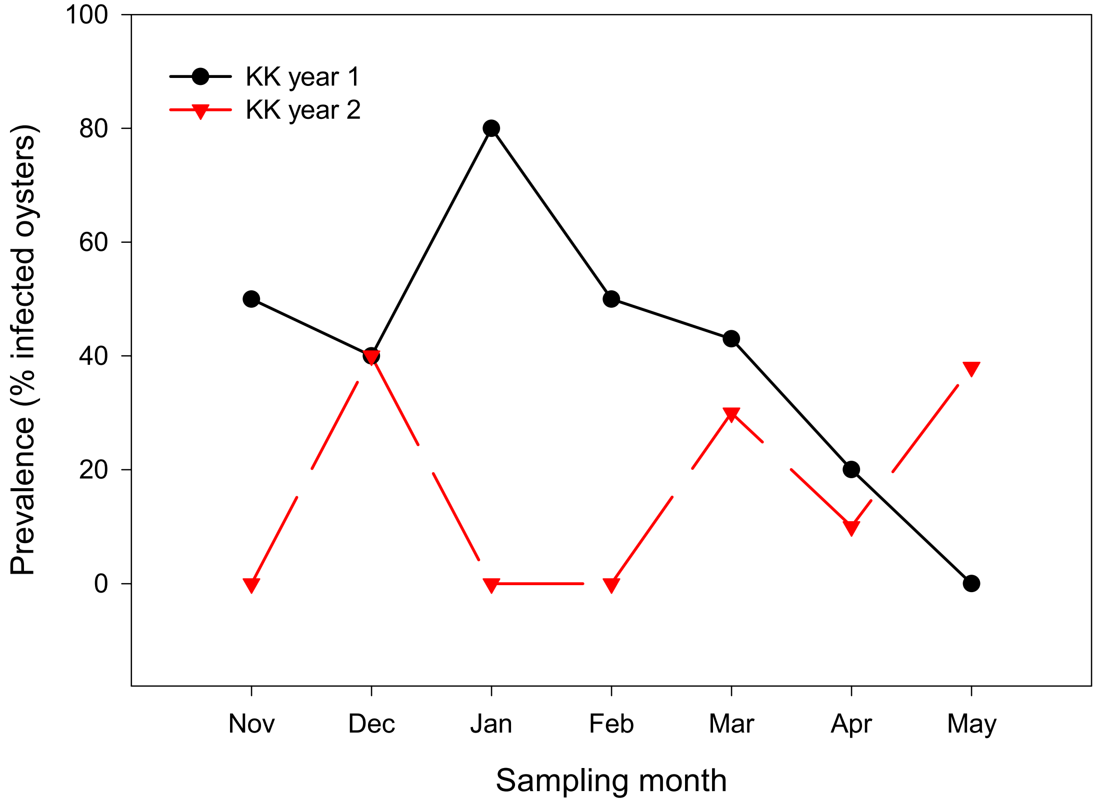

Fig. 13: Mean *P. marinus* prevalence (± SE) during winter months in oysters from Kitchel Key (KK) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters, however, due to the proximity of this station to marine environment and higher salinities, effects of freshwater releases are less pronounced.

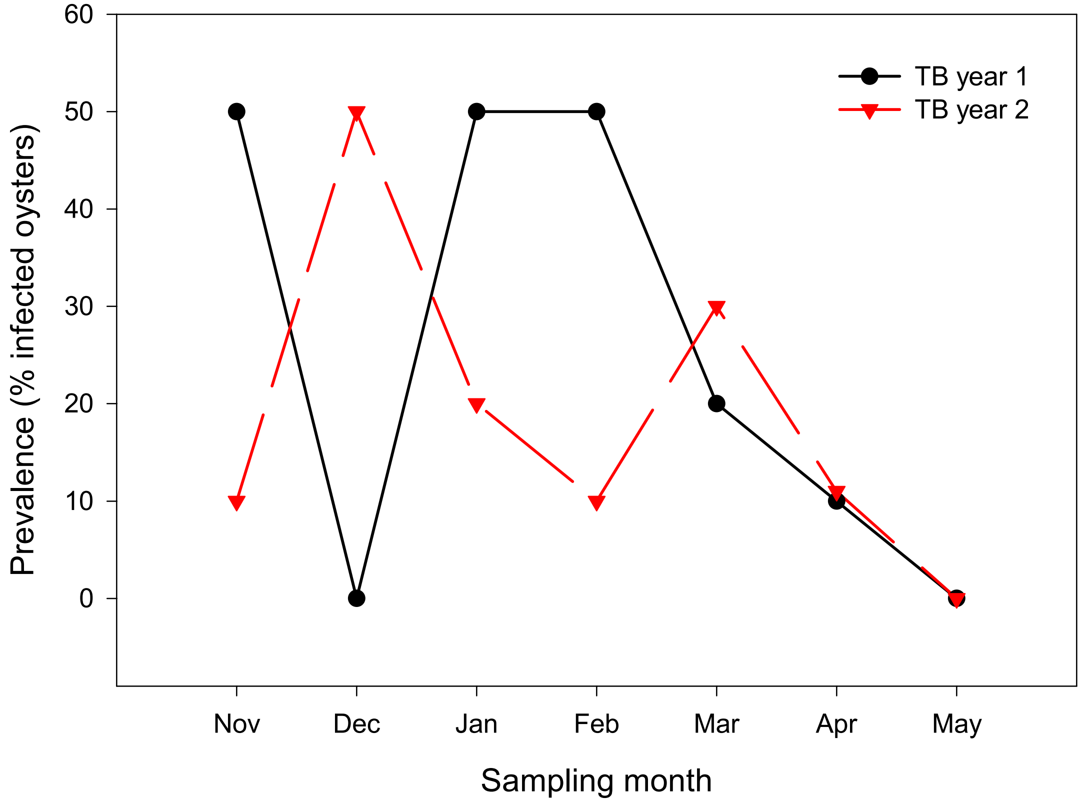

Fig. 14: Mean *P. marinus* prevalence (± SE) during winter months in oysters from Tarpon Bay (TB) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters, however, due to the proximity of this station to marine environment (downstream of river) and higher salinities, effects of freshwater releases are less pronounced.

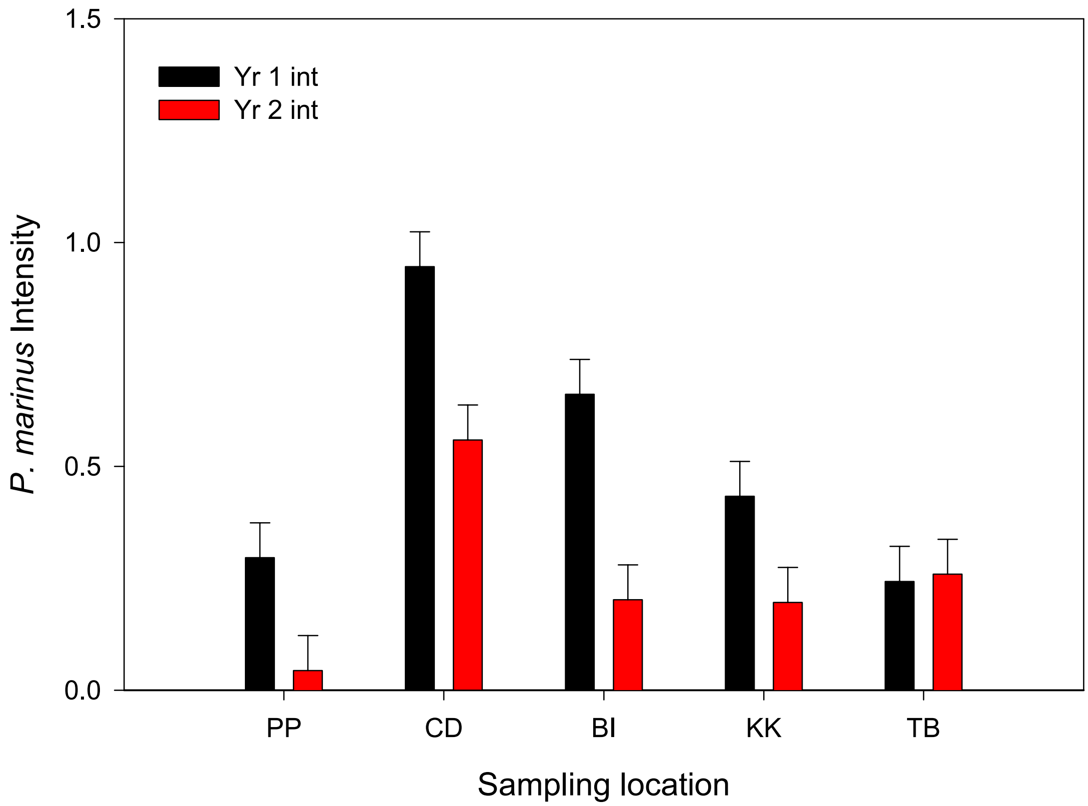

Fig. 15: Mean *P. marinus* intensity (± SE) during winter months in oysters from Piney Point (PP), Cattle Dock (CD), Bird Island (BI), Kitchel Key (KK), and Tarpon Bay (TB) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Ten oysters per month were randomly samples from the sampling locations per month and intensity (Int) of *P. marinus* (weighted incidence) was analyzed according to Ray 1954. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities resulted in lower infection intensities in oysters from all upstream locations.

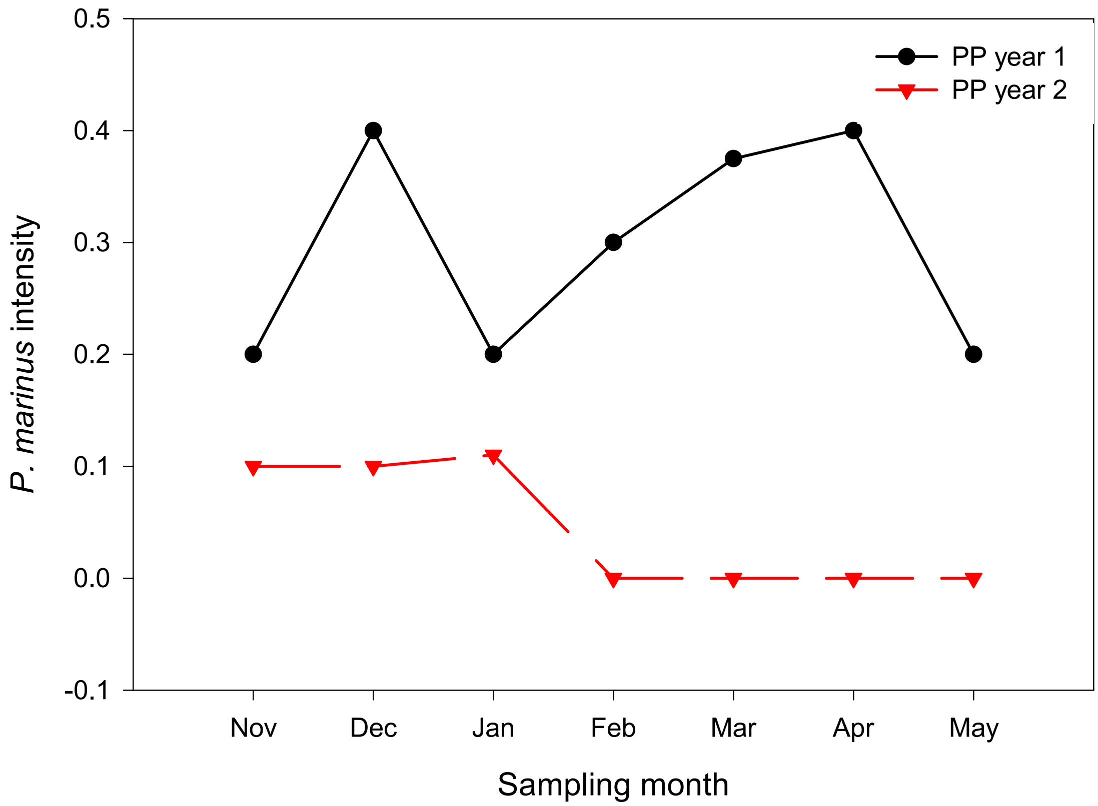

Fig. 16: Mean *P. marinus* intensity during winter months in oysters from Piney Point (PP) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters.

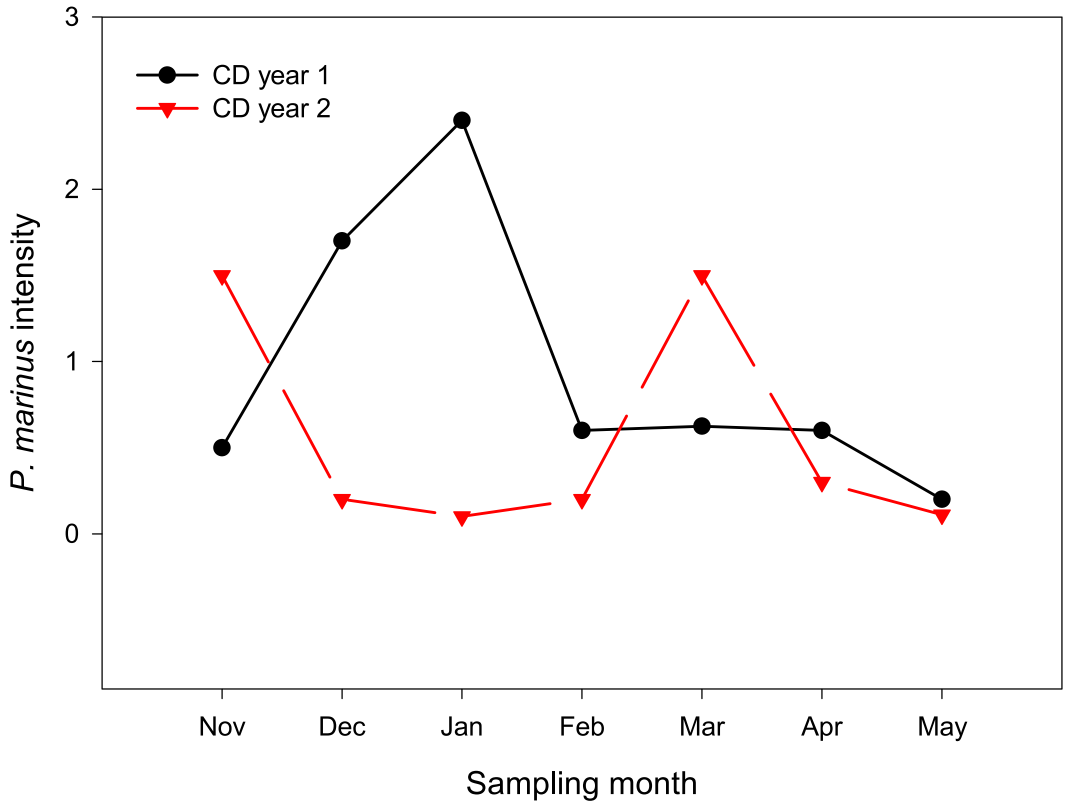

Fig. 17: Mean *P. marinus* intensity during winter months in oysters from Cattle Dock (CD) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters. Cattle Dock site also receives runoff water from the City of Cape Coral and nearby marinas.

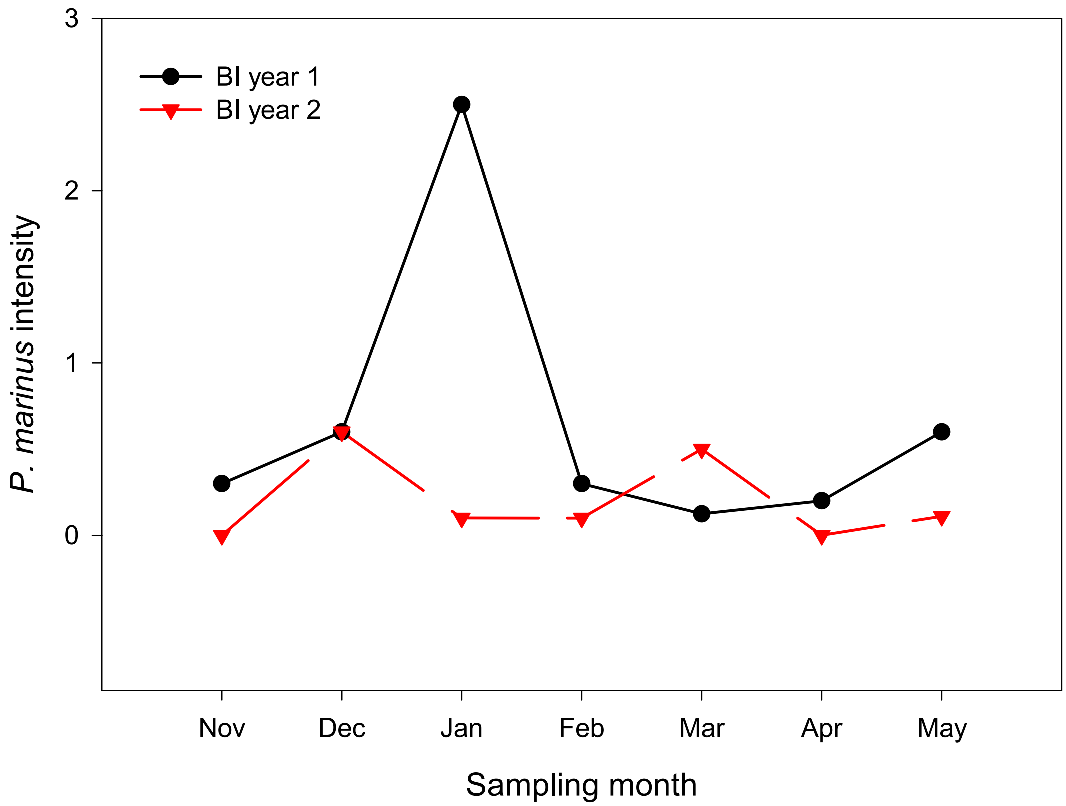

Fig. 18: Mean *P. marinus* intensity during winter months in oysters from Bird Island (BI) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters.

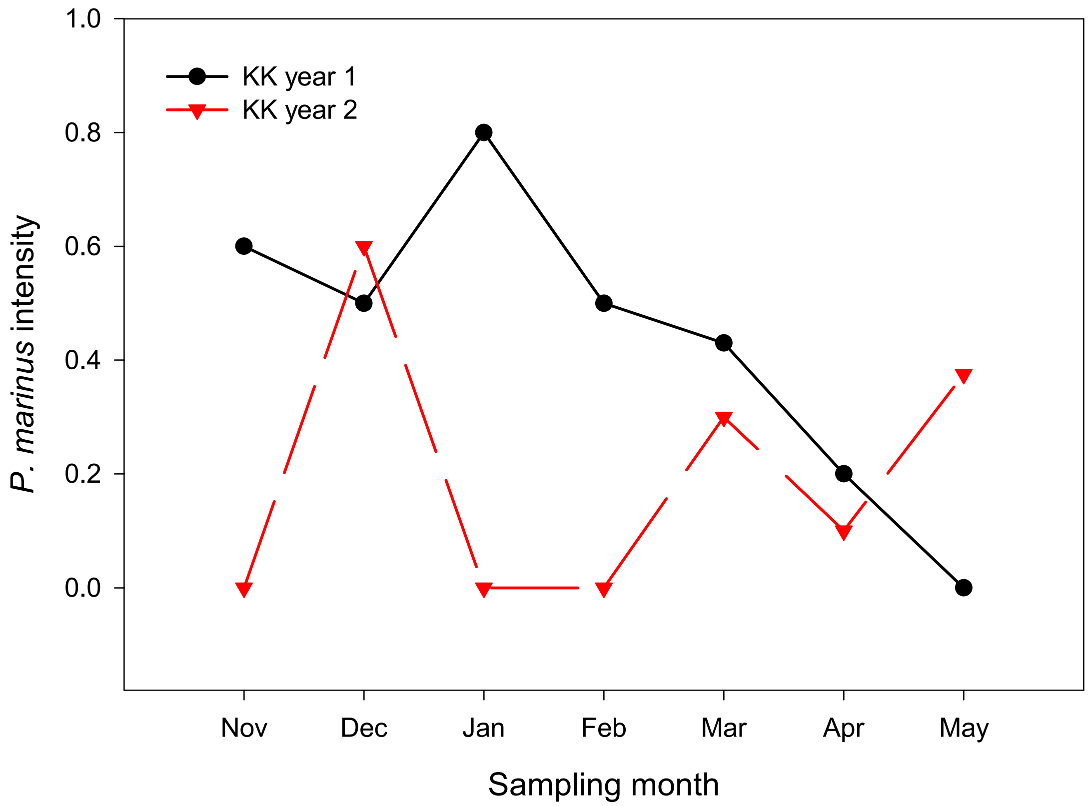

Fig. 19: Mean *P. marinus* intensity during winter months in oysters from Cattle Dock (CD) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters.

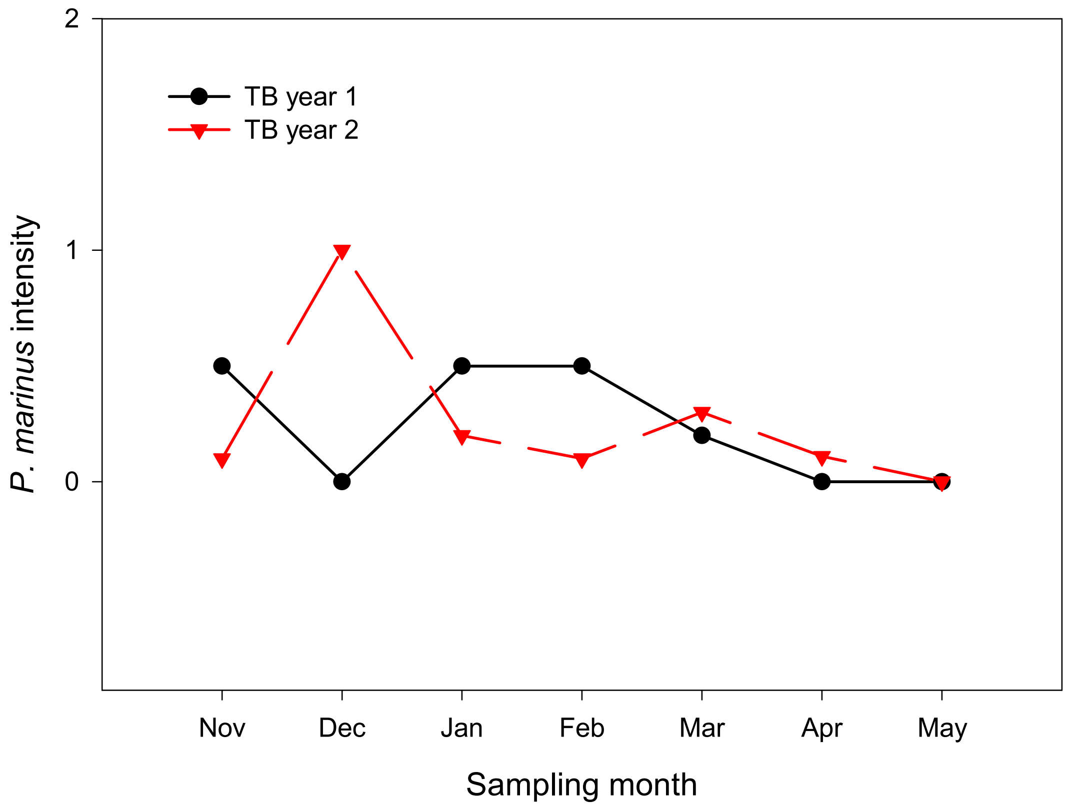

Fig. 20: Mean *P. marinus* intensity during winter months in oysters from Tarpon Bay (TB) in Caloosahatchee River during years 1 and 2. November - May were considered as dry months due to the paucity of rainfall. Years 1 and 2 are from September 2000 - August 2001, and from September 2001 - Present, respectively. Increased freshwater releases from Lake Okeechobee and resulting decreased salinities during year 2 resulted in lower prevalence of *P. marinus* infections in oysters, however, due to the proximity of this station to marine environment (downstream of river) and higher salinities, effects of freshwater releases are less pronounced.